LOW–TEMPERATURE, LONG–TIME HEATING OF BOVINE MUSCLE
2. Changes in Electrophoretic Patterns

SUMMARY—Polyacrylamide gel electrophoresis was used to follow changes in the nature of the water-soluble proteins and juices of bovine muscle during low-temperature heating. The slowest-moving anodic proteins were coagulated first. The myoglobins and myoalbumins were altered significantly only by holding the meat at 60°C. The largest changes in tenderness and amount of water-soluble material in the meat occurred at the same temperatures under which the slow-moving proteins were denatured. The most heat-sensitive proteins detected were denatured before there were significant changes in tenderness or water-soluble substance content.

INTRODUCTION

RANDALL et al. (1967) found 3 cathodic bands and 13 anodic bands using starch gel electrophoresis of the water-soluble fraction of bovine skeletal muscle. Scopes (1964) has shown that myoalbumin is the fastest-moving anodic band in sarcoplasmic extracts. Quinn et al. (1964a; 1964b) found at least 3 myoglobin bands, the slowest being the most predominant. Randall et al. (1967) observed 2 pigmented bands, the 6th and 7th from the anodic end, the faster being the more intensely colored. Rowland et al. (1968) found 1 major myoglobin band and several more rapid forms using polyacrylamide gel electrophoresis.

Most of the sarcoplasmic proteins coagulate when bovine muscle reaches 40–60°C (Hamm, 1966). Grau et al. (1963) showed that the proteins migrating in an electric field with the greatest velocity were denatured the most easily, although the cathodic proteins were more stable (Lee et al., 1966). The juice from cooked meat shows different electrophoretic patterns from the cooked juice of raw meat, which Lee et al. (1966) suggested was due to an influence of the myofibrillar proteins. Quinn et al. (1964a; 1964b) found that the 3 myoglobins evident in their electrophoretic patterns were not affected by heating to 55°C for 5 min.

The objective of this study was to relate changes in the electrophoretic patterns of water-soluble proteins and juices obtained from bovine muscle during low-temperature cooking to changes observed in the tenderness, water-holding capacity, pH and amount of water-soluble protein.

EXPERIMENTAL

LONGISSIMUS, rectus femoris and semitendinosus muscles from Hereford steers were heated at 0.1°C/min to holding temperatures of 37, 45 and 60°C. Samples were withdrawn periodically during the heating program for the various measurements. The freeze-dried water extracts were used for the electrophoresis. Details on muscle preparation, heating program and extraction of the water-soluble material were presented in the preceding paper (Laakkonen et al., 1969).

The method for vertical polyacrylamide gel electrophoresis presented by Thompson et al. (1964) was modified for this study. The gels were 6% in polyacrylamide and 4.5 M in urea. The electrophoresis cell was buffered to pH 8.2. Gel formation was catalyzed by addition of 0.2% ammonium persulfate. 40 mg of the freeze-dried water-soluble fraction was dissolved in 0.5 ml distilled water, the solution saturated with sucrose and 2 drops of bromphenol blue added. Electrophoresis was done at 6°C for 7 hr. The initial current was set to 50 mA and increased 5 mA every 15 min until the voltage reached 250 v. The voltage was then kept constant to the end of the run. The gels were stained with Amido Black 10B and destained with 7% acetic acid. They were stored in the destaining solution until photographed. Histograms were constructed such that the width of the bar indicates the width of the band and the height indicates the estimated intensity. The distance migrated relative to the front is indicated by the position of the bar on the abscissa.

RESULTS

AS EXPECTED, the electrophoretic patterns of the water-soluble material from raw meat shown at the top of Figure 1 are similar to those obtained by Randall

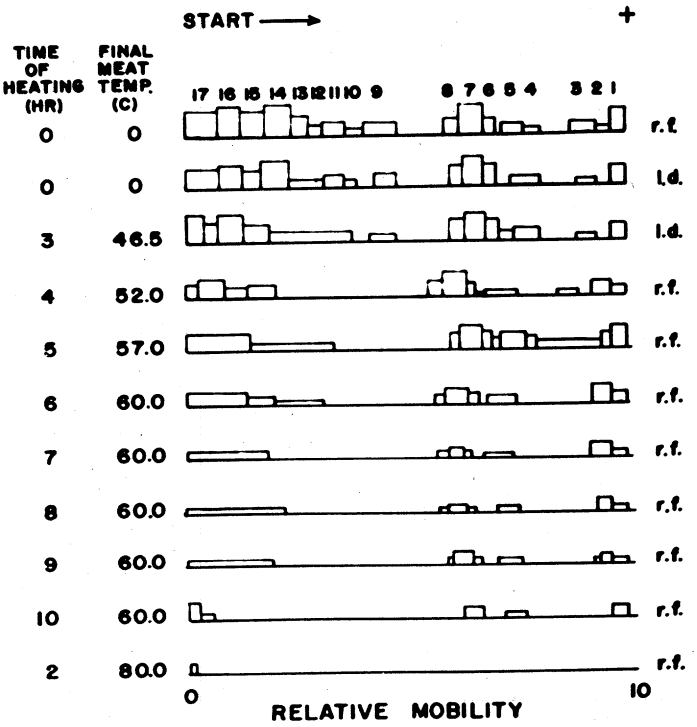

Fig. 1—Electrophoretograms of freeze-dried water-soluble proteins from samples heated to 60°C at 0.1°C/min and held for 10 hr total heating time. Muscles: l.d. = longissimus, r.f. = rectus femoris. Control: rectus femoris heated to 80°C.

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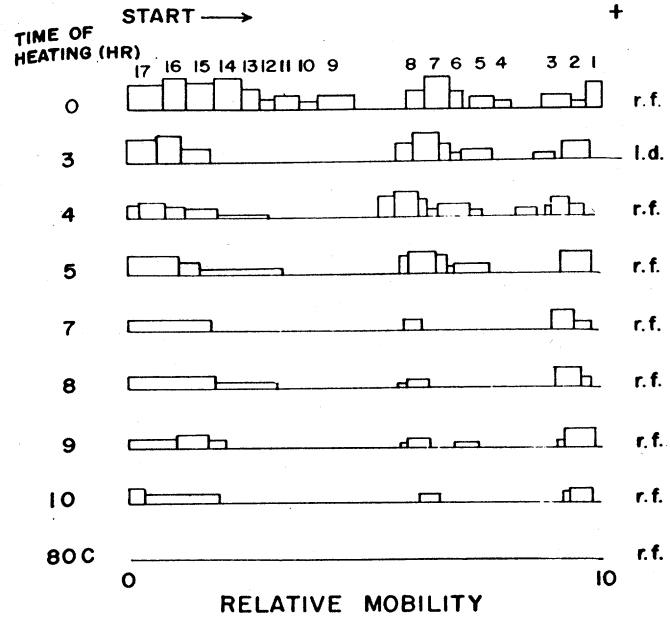

Fig. 2.—Electrophoretograms of freeze-dried meat juices (drip) exuded from samples heated to 60°C at 0.1°C/min and held for 10 hr total heating time. Muscles: l.d. = longissimus, r.f. = rectus femoris. Control: rectus femoris heated to 80°C. The pattern for water-soluble proteins from unheated rectus femoris is included for reference.

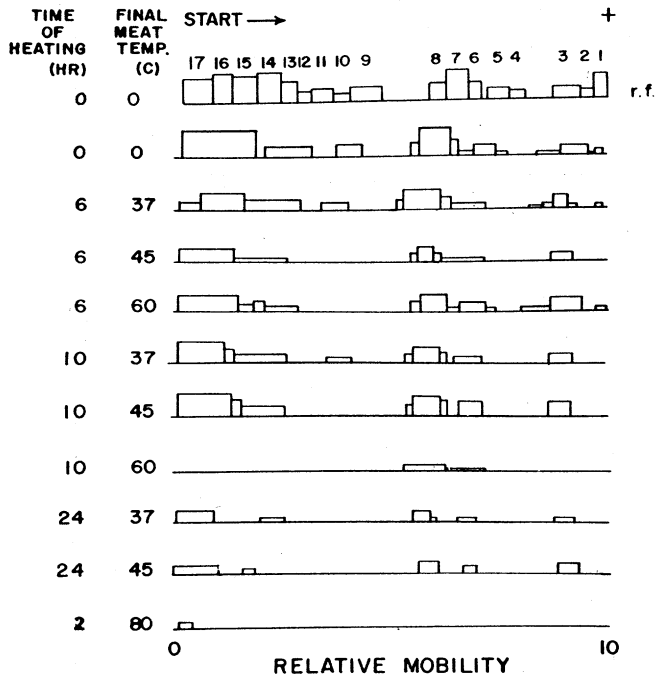

Fig. 3.—Electrophoretograms of freeze-dried water-soluble proteins from samples heated to 37, 45 and 60°C at 0.1°C/min and 80°C at 0.8°C/min. Muscle: longissimus (r.f. = rectus femoris shown for reference).

let al. (1967). Although 17 anodic bands are present, the higher pH used in the present study may have changed some proteins from a net positive to a net negative charge. The 5 slowest-moving bands were difficult to differentiate, but they included 2 intensively stained zones, bands 14 and 16. Band 7 had the most intense meat color prior to staining, but band 8 was also colored. Bands 5 and 6 were more weakly colored. This agrees with the results of Quinn et al. (1964a; 1964b) and Rowland et al. (1968). In fact, it confirms Quinn's postulation of 4 myoglobin bands. Sharp bands were not always seen, but stained areas are depicted in the figures since they disappeared with continued heating.

Figure 1 shows the changes in the electrophoretic patterns during heating. Bands 9–11 were the most heat sensitive and could not be extracted from muscle heated for 4 hr (50.5°C in the center and 52.0°C on the surface). Bands 14–17, the slowest-moving, started to blur and disappear from extracts of meat cooked for 5 hr. The myoglobin bands, 5–8, appeared to remain unchanged until the holding temperature of 60°C had been reached. Following this, the amounts of myoglobins decreased gradually for the rest of the holding time. The myoalbumins, bands 1–3, were not affected until the meat reached 52°C. Band 3 was diffuse in extracts of meat heated to 57°C, then disappeared. In raw and partially rare meat, band 1 was more intense than band 2. At 60°C, this was reversed. Extracts from the control sample, heated to 80°C in 1 hr and held 1 hr, had only a trace of protein-like material and that did not leave the origin.

Figure 2 shows the changes in the proteins of the meat juice (drip) exuded from the samples. Below 45°C, the amount of juice was very small, as reported in the preceding paper. The most heat-sensitive bands, 9–11, did not appear in these electrophoretograms. The slowest group of proteins was more clearly defined and unchanged in the juice

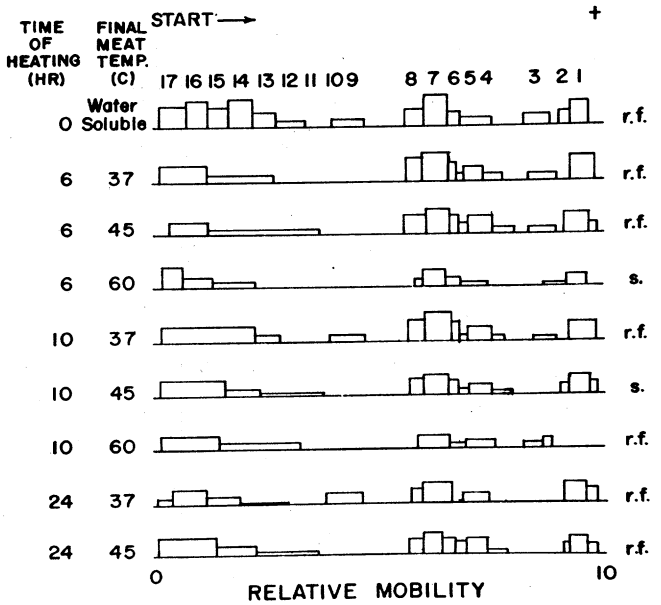

Fig. 4.—Electrophoretograms of the freeze-dried juices (drip) exuded from samples heated to 37, 45 and 60°C at 0.1°C/min and 80°C at 0.8°C/min. Muscles: r.f. = rectus femoris, s = semitendinosus. The pattern for water-soluble proteins from unheated rectus femoris is included for reference.

than in the extracts. After 7 hr of cooking, the myoglobin bands were less intense and less clearly resolved in the juice than in the water extracts. The juice appeared to have a slightly higher concentration of the myoalbumin group. No mobile proteins were found in the juice from the control sample. The difference between the electrophoretograms of meat juice and of water-soluble material from cooked meat is clear but small, as stated by Lee et al. (1966).

Figures 3 and 4 show the electrophoretograms of water-soluble proteins and juices, respectively, of meat samples held at different temperatures. Band 11 could not be seen in any of the heated samples, but band 9 was detectable in the meat held at 37°C. The myoglobins and myoalbumins were little affected by holding the meat at temperatures below 60°C.

Loss of solubility of the slowest-moving proteins occurs at the same points in the heating schedule as do the largest decreases in shear values and total amount of water-soluble material reported in our first paper. During the 60°C holding period, shear values and content of water-soluble material remained essentially constant in spite of the apparent decrease in extractable myoglobins. The weight loss of the meat does not seem to be related to the electrophoretically observable changes. There did seem to be fewer extractable proteins in samples having the higher weight loss.

REFERENCES

Grau, R. and Lee, F.A. 1963. Über den Einfluss der Temperatur auf das Verhalten der Eiweisstoffe des Rindermuskels. Naturwissenchaften 50: 379.

Hamm, R. 1966. Heating of muscle systems. In "The Physiology and Biochemistry of Muscle as a Food," eds. Briskey, E.J., Cassens, R.G. and Trautman, J.C. pp. 363-385. University of Wisconsin Press, Madison.

Laakkonen, E., Wellington, G.H. and Sherbon, J.W. 1970. Low-temperature long-time heating of bovine muscle. I. Changes in tenderness, water-binding capacity, pH and the amount of water-soluble components. J. Food Sci. 35: 175-177.

Lee, F. and Grau, R. 1966. Verhalten von Rindersarkoplasm beim Erhitzen. (On the influence of temperature on the behavior of soluble proteins of beef.) Fleischwirtschaft 46: 1239.

Quinn, J.R., Pearson, A.M. and Brunner, J.R. 1964a. Detection and isolation of multiple myoglobins from beef muscle. J. Food Sci. 29: 422.

Quinn, J.R. and Pearson, A.M. 1964b. Characterization studies of three myoglobin fractions from bovine muscle. J. Food Sci. 29: 429.

Randall, C.J. and MacRae, H.F. 1967. Hydrolytic enzymes in bovine skeletal muscle. II. Proteolytic activity of the water-soluble proteins separated by starch gel electrophoresis. J. Food Sci. 32: 182.

Rowland, L.P., Dunne, P.B., Penn, A.S. and Maher, E. 1968. Myoglobin and muscular dystrophy. Arch. Neurol. 18: 141. [C.A. 68, (15): 6723d, 1968.]

Scopes, R.K. 1964. The influence of post-mortem conditions on the solubilities of muscle proteins. Biochem. J. 91: 201.

Thompson, M.P., Kiddy, C.A., Johnston, J.O. and Weinberg, R.M. 1964. Genetic polymorphism in caseins of cows' milk. II. Confirmation of the genetic control of β-casein. J. Dairy Sci. 47: 378.

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